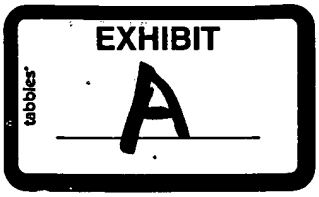

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/990/087
Confirmation No.:		1280
Applicant	:	Sligar et al.
Filed	:	November 20, 2001
TC/A.U.	:	1646
Examiner	:	Ruixian LI
For	:	MEMBRANE SCAFFOLD PROTEINS
Docket No.	:	87-00
Customer No.	:	23713

DECLARATION OF DANIEL OPRIAN, Ph.D.

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Daniel Oprian, Ph.D. declare as follows:

1. I am the Louis and Bessie Rosenfield Professor of Biochemistry at Brandeis University.

2. I received a Ph.D. in Biochemistry from the University of Michigan in 1980.

3. A copy of my curriculum vitae is attached hereto as is a list of my publications.

4. My primary research interest involves studies of the structure and function of G protein-coupled receptors.

5. I am making this declaration for the purpose of providing my scientific opinion that the incorporation of cytochrome P450 reductase (a single pass membrane protein) into a disc consisting of phospholipids and a natural apoliprotein A1 scaffold protein does not provide evidence that one would be able to incorporate a protein having seven transmembrane domains such as a G protein-coupled receptor (GPCR) into a disc

consisting of a membrane scaffold protein and phospholipid while maintaining its ability to bind ligand.

6. I have reviewed Bayburt et al., "Reconstituting and Imaging of a Membrane Protein into a Nanometer-Size Phospholipid Bilayer," *J. Struc. Biol., 123, 37-44 (1998)* which describes inserting cytochrome P450 reductase into a phospholipid bilayer stabilized by naturally occurring apoliprotein A1. The authors demonstrated that they were able to insert the P450 reductase into the bilayer and that the reductase retained its catalytic activity.

7. Cytochrome P450 reductase has a single transmembrane alpha helical domain which passes once through the phospholipid bilayer and with the hydrophobic face of the alpha helix oriented outward to interact with the phospholipid in the membrane. The reductase retains certain of its activities even when not associated with a membrane.

8. In contrast, a G protein-coupled receptor (GPCR) has a significantly more complicated transmembrane structure which consists of seven transmembrane alpha helical segments each of which must be properly oriented with respect to the phospholipids (*i.e.*, hydrophobic surface facing the phospholipid) and which must be properly oriented with respect to each other and which must be oriented such that certain hydrophilic portions of the GPCR reside outside of the phospholipid. *In vivo* this is accomplished during biosynthesis of GPCRs, when the protein is processively inserted in to the membrane in an ordered-fashion which results in the proper helix-lipid and helix-helix organization and folding of the protein within the membrane that allows it to have its appropriate ligand binding ability.

9. Based on the significantly more complicated helix-helix and helix-phospholipid interactions required for proper folding and orientation of GPCRs in a membrane, the demonstration that a simple single pass membrane protein such as cytochrome P450 reductase can be inserted into a phospholipid bilayer does not provide proof that an isolated GPCR could also be successfully inserted into such a bilayer and be properly folded and oriented to maintain ligand binding ability.

10. The successful insertion (such that natural ligand binding and coupling to G-proteins is preserved) of GPCRs and other seven transmembrane proteins in discs comprising modified membrane scaffold proteins and phospholipids such as those described in the above-identified patent application also helps fill a need for materials and methods for studying and utilizing GPCRs which were unavailable until the demonstration by Dr. Stephen Sligar and his colleagues that such discs could be made.

11. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/14/05
Date

Daniel Oprian, Ph.D.

Curriculum Vitae

Daniel D. Oprian

Louis and Bessie Rosenfield Professor of Biochemistry

Education

University of Michigan	BS 1975	Cellular Biology
University of Michigan Medical School	PhD 1980	Biological Chemistry

Professional Positions

Postdoctoral Fellow	U of M Medical School	1980-82
Postdoctoral Fellow	MIT	1982-87
Assistant Professor	Brandeis University	1987-93
Associate Professor	Brandeis University	1993-96
Louis and Bessie Rosenfield Professor of Biochemistry	Brandeis University	1996-
Chairman, Biochemistry Dept.	Brandeis University	1996-2005

Other Professional Activities

NIH Study Section, Ad Hoc	1995
Member, NIH Study Section (VISC)	1996-2001
Editorial Advisory Board, Biochemistry	1997-
Consultant, Proctor & Gamble Pharmaceuticals	1999-2000
Co-Chair, FASEB Summer Research Conference on The Biology and Chemistry of Vision	1997
Chair, FASEB Summer Research Conference on The Biology and Chemistry of Vision	1999
Co-Chair, International Symposium on Nucleic Acids and Signal Transduction	2000
Ad Hoc Member, BSC NIDCD	2000
Session Chair, Pfizer Symposium on GPCRs	2000
Outside examiner, PhD Thesis, Christian E. Elling, University of Copenhagen, Denmark	2000
Symposium Chair, Biophysical Society Meeting	2001
Ad Hoc Member, BSC NIDCD	2004
International Organizing Committee, Symposium on Nucleic Acids, Membranes, and Signal	

Transduction 2004

Membership in Professional Societies

American Society for Biochemistry and Molecular Biology
Biophysical Society
AAAS

Honors and Awards

Plenary Lecture, FASEB Conference on Retinoids, Saxtons River, VT, 1992
Raiziss Lecture, University of Pennsylvania School of Medicine, 1995
Plenary Lecture, FASEB Conference on The Biology and Chemistry of Vision, 1995
Louis and Bessie Rosenfield Professor of Biochemistry, Brandeis University, 1996
University of Michigan Distinguished Graduate, Ann Arbor, MI, 2000

Invited Lectures

(the following list is incomplete because of gaps in my records for years 88-92)

1986 University of Texas Southwestern Medical Center, Dallas
University of Wisconsin, Madison
University of Colorado, Boulder
University of California, Santa Barbara
Washington University, St. Louis
1987 Princeton University
Brandeis. University
Purdue University
Rice University
University of Virginia, Charlottesville
University of St. Louis
1989 FASEB Conference on The Biology and Chemistry of Vision
1991 Cleveland Clinic
Tufts University
Lecture in Neuroscience course, MBL, Woods Hole, MA
FASEB Conference on The Biology and Chemistry of Vision
1992 Boston University Medical School
Harvard Medical School, Mass Eye and Ear
Stanford University
University of California, San Francisco
University of Connecticut, Hartford
University of California, Los Angeles
University of Washington, Neuroscience Symposium
Beckman Symposium, Receptor Proteins: Structure, Function, and Modeling, Urbana

Plenary Lecture, FASEB Conference on Retinoids, Saxtons River, VT
20th Meeting of American Society for Photobiology, Marco Il., FL
Vth International Conference on Retinal Proteins, Dourdan, France
Genentech, San Francisco
Discussion Leader, "Controversies in Neuroscience III: Signal Transduction in the Retina and Brain", Portland, OR
Yale University, MB&B
18th Taniguchi Symposium on Biophysics, Kyoto

1993 Serono Symposium On Inflammation, Heidelberg
Gordon Conference on Second Messengers
FASEB Conference on The Biology and Chemistry of Vision
Gordon Conference on Bioenergetics
Harvard University
National Institutes of Health, NEI
Tufts University

1994 Glaxo
Keystone Symposium, Signal Transduction
Biophysical Society Meeting, New Orleans
Boston University
VIth International Conference on Retinal Proteins, Leiden, The Netherlands
Regional ACS Meeting, Ann Arbor, MI
FASEB conference on Membrane Molecular Biophysics, Saxtons River, VT
University of Michigan
University of Virginia, Charlottesville
Tufts University School of Medicine
State University of New York, Buffalo
University of Colorado, Boulder
Harvard Medical School

1995 Boston University Medical School
Raiziss Lecture, University of Pennsylvania School of Medicine
Vollum Institute, Oregon Health Sciences University
Alfred Benzon Symposium on the Structure and Function of 7TM Receptors, Copenhagen
Plenary Lecture, FASEB Conference on The Biology and Chemistry of Vision
American Chemical Society Meeting, Chicago
Mount Sinai School of Medicine, New York
University of Washington, Seattle
University of California, Berkeley

1996 Bristol-Meyers/Squibb (Wallingford)
Yale University, MB&B
Gordon Conference on Chemokines, Chairman of Receptor Session, Holderness, NH
FASEB Conference on Membranes, Saxtons River, VT

1997 Presenilin Conference, Salk Institute, La Jolla, CA
Gordon Conference on Ligand Recognition and Molecular Gating, Plymouth, NH

1998 UMass, Amherst
ARVO (Association for Research in Vision and Ophthalmology) Meeting, Ft. Lauderdale, FL

University of California, Los Angeles
1999 Symposium in Honor of George Wald, ARVO, Ft. Lauderdale, FL
NIST, Rockville, MD
Chemistry Dept., Boston College, Newton, MA
2000 Harvard, Mass Eye and Ear, Boston, MA
Dept. Cell Biology, Med. College of Wis., Milwaukee, WI
University of Michigan Distinguished Graduate, Ann Arbor, MI
Physiology course, Marine Biological Laboratories, Woods Hole, MA
SUNY at Albany, Albany, NY
University of Copenhagen, Denmark
2001 Biophysical Society Meeting, Boston, MA
Keystone Symposium on Membrane Protein Structure/Function Relationships, Tahoe City, CA
University of CT, Storrs, CT
Physiology course, MBL, Woods Hole, MA
FEBS-EMBO advanced lecture course on Molecular Mechanisms in Signal Transduction,
Spetses, Greece
University of Michigan, Ann Arbor, MI
2002 Gordon Research Conference on Ligand Recognition and Molecular Gating, Barga, Italy
10th International Conference on Retinal Proteins, Seattle, WA
University of Vermont, Burlington, VT
2003 Tufts School of Medicine, Boston
7th Annual Vision Research Conference, Fort Lauderdale, FL
MRC-LMB, Cambridge, UK
The Physiological Society, Cambridge, UK
2004 Symposium on Nucleic Acids, Membranes, and Signal Transduction, Okayama, Japan
Boston University School of Medicine, Boston, MA
2005 Volen Center Retreat, Brandeis University, Waltham. MA

Research Projects Ongoing or Completed During the Last 3 Years

"Structure-Function Studies of Rhodopsin"
Principal Investigator: Daniel D. Oprian
Agency: National Eye Institute
5 RO1 EY 07965 Period: 12/1/2003 – 11/30/2008
The major goal of this project is to elucidate the mechanism of action of the visual pigment rhodopsin.

"Mechanisms of Spectral Tuning"
Principal Investigator: Daniel D. Oprian
Agency: National Eye Institute
2 RO1 EY09514 Period: 5/1/01 – 4/30/05

The major goal of this project is to elucidate the molecular mechanisms underlying spectral tuning in the human color vision pigments with emphasis on the short wave-group including the human blue pigment and vertebrate ultraviolet receptors.

"Structure of the Ligand Binding Domains of the CCR5 Receptor"
Project #4 of Program Project: Structure and combinatorial Chemistry in HIV Drug Design
Principal Investigator: Stephen C. Harrison, Program Director)
Agency: National Institute of General Medicine
2 PO1 GM 39589 Period: 7/1/98 – 6/30/03
The major goals of this project are: (1) To systematically map tertiary contacts in the CCR5 receptor and build a three dimensional map of the protein; (2) to establish high level expression and purification of CCR5 for the purpose of attempting 2- and 3-dimensional crystallization of CCR5 alone and in complexes with CD4 and HIV env.

"The Molecular Mechanism of G Protein-Couple Receptor Signaling"
Co-Investigators: Gebhard Schertler, Daniel D. Oprian, and Christian Riekel
Agency: Human Frontier Science Program
Program Project RGP0052/2005-C Period: 2005 - 2008
The goal of this project is to determine a structure for an activated GPCR/target complex using (and developing) state-of-the-art crystallization and diffraction technologies for small crystals.

University Service

Adjudication Committee, 1995-2001
Ad Hoc Promotion Committees
Life Sciences Committee
Freshman Advisor

Departmental Service

Graduate Admissions Committee
Space Committee
Graduate Curriculum Committee
Life Sciences Colloquium Series

Students and Postdoctoral Fellows

Undergraduate (honors and MS theses)
Allan Pearson
Asli Kumbasar
Leah Friedman
Mehesh Karandekar

Doctoral
Eugene Zhukovsky
Jancy McPhee
George Cohen
Sandra Pelletier
Ana Asenjo
Ning Lee
Vikram Rao
Zhiyan Wang
Jeanne Rim
Tong Yang
Hongbo Yu
Tim Strassmaier
Tim McKee
Shengnan Jin
Alecia Gross
Adam Andrew
Amy Swift

Postdoctoral Fellows
Phyllis Robinson
Chandrika Govardhan
Mas Kono
Mary Struthers
Jeff Fasick
Patrizia Hagenbuch
Yagya Sharma
Annette Sievers

Courses Taught

Undergraduate
BCSC 7b Drug Discovery and Development
CHEM 25b Organic Chemistry
BCHM 100a Biochemistry

Graduate
BCHM 101a Advanced Biochemistry I, chemistry of enzyme active sites
BCHM 101b Advanced Biochemistry II, chemical logic of metabolic pathways, membrane protein structure and function, mechanisms in signal transduction
Advanced Topics Courses:
Molecular Biology of Vision
Biochemical Signaling Mechanisms
Membrane Protein Structure and Function

Publications:

Coon, M. J., Blake, R. C., 2nd, Oprian, D. D., and Ballou, D. P. Mechanistic studies with purified components of the liver microsomal hydroxylation system: spectral intermediates in reaction of cytochrome P-450 with peroxy compounds. (1979) *Acta Biol Med Ger* **38**, 449-58.

Vatsis, K. P., Oprian, D. D., and Coon, M. J. Kinetics of reduction of purified liver microsomal cytochrome P-450 in the reconstituted enzyme system studied by stopped flow spectrophotometry. (1979) *Acta Biol Med Ger* **38**, 459-73.

Oprian, D. D., Vatsis, K. P., and Coon, M. J. Kinetics of reduction of cytochrome P-450LM4 in a reconstituted liver microsomal enzyme system. (1979) *J Biol Chem* **254**, 8895-902.

Oprian, D. D., and Coon, M. J. Oxidation-reduction states of FMN and FAD in NADPH-cytochrome P-450 reductase during reduction by NADPH. (1982) *J Biol Chem* **257**, 8935-44.

Oprian, D. D., Gorsky, L. D., and Coon, M. J. Properties of the oxygenated form of liver microsomal cytochrome P-450. (1983) *J Biol Chem* **258**, 8684-91.

Yatsunami, K., Pandya, B. V., Oprian, D. D., and Khorana, H. G. cDNA-derived amino acid sequence of the gamma subunit of GTPase from bovine rod outer segments. (1985) *Proc Natl Acad Sci U S A* **82**, 1936-40.

Barrett, D. J., Redmond, T. M., Wiggert, B., Oprian, D. D., Chader, G. J., and Nickerson, J. M. cDNA clones encoding bovine interphotoreceptor retinoid binding protein. (1985) *Biochem Biophys Res Commun* **131**, 1086-93.

Ferretti, L., Karnik, S. S., Khorana, H. G., Nassal, M., and Oprian, D. D. Total synthesis of a gene for bovine rhodopsin. (1986) *Proc Natl Acad Sci U S A* **83**, 599-603.

Oprian, D. D., Molday, R. S., Kaufman, R. J., and Khorana, H. G. Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. (1987) *Proc Natl Acad Sci U S A* **84**, 8874-8.

Franke, R. R., Sakmar, T. P., Oprian, D. D., and Khorana, H. G. A single amino acid substitution in rhodopsin (lysine 248----leucine) prevents activation of transducin. (1988) *J Biol Chem* **263**, 2119-22.

Zhukovsky, E. A. and Oprian, D. D. Effect of Carboxylic Acid Side Chains on the Absorption Maximum of Visual Pigments. (1989) *Science* **246**, 928-930.

Zhukovsky, E. A., Robinson, P. R., and Oprian, D. D. Transducin Activation by Rhodopsin Without a Covalent Bond to the 11-*Cis*-Retinal Chromophore. (1991) *Science* **251**, 558-560.

Oprian, D. D., Asenjo, A. B., Lee, N., and Pelletier, S. L. Design, Chemical Synthesis, and Expression of Genes for the Three Human Color Vision Pigments. (1991) *Biochemistry* **30**, 11367-11372.

Oprian, D. D. The Ligand-Binding Domain of Rhodopsin and Other G Protein-Linked Receptors. (1992) *J. Bioenerg. Biomem.* **24**, 211-217.

Oprian, D. D. Molecular Determinants of Spectral Properties and Signal Transduction in the Visual Pigments. (1992) *Curr. Opin. Neurobiol.* **2**, 428-432.

Robinson, P. R., Cohen, G. B., Zhukovsky, E. A., and Oprian, D. D. Constitutively Active Mutants of Rhodopsin. (1992) *Neuron* **9**, 719-725.

Zhukovsky, E. A., Robinson, P. R., and Oprian, D. D. Changing the Location of the Schiff Base Counterion in Rhodopsin. (1992) *Biochemistry* **31**, 10400-10405.

Cohen, G. B., Oprian, D. D., and Robinson, P. R. Mechanism of Activation and Inactivation of Opsin: Role of Glu^{113} and Lys^{296}. (1992) *Biochemistry* **31**, 12592-12601.

Wang, Z., Asenjo, A. B., and Oprian, D. D. Identification of the Cl^--Binding Site in the Human Red and Green Color Vision Pigments. (1993) *Biochemistry* **32**, 2125-2130.

Dryja, T. P., Berson, E. L., Rao, V. R., and Oprian, D. D. Heterozygous Missense Mutation in the Rhodopsin Gene as a Cause of Congenital Stationary Night Blindness. (1993) *Nature Genetics* **4**, 280-283.

Oprian, D. D. Expression of Opsin Genes in COS Cells. (1993) *Methods Neurosci.* **15**, 301-306.

Cohen, G. B., Yang, T., Robinson, P. R., and Oprian, D. D. Constitutive Activation of Opsin: Influence of Charge at Position 134 and Size at Position 296. (1993) *Biochemistry* **32**, 6111-6115.

Govardhan, C. P. and Oprian D. D. Active-Site Directed Inactivation of Constitutively Active Mutants of Rhodopsin. (1994) *J. Biol. Chem.* **269**, 6524-6527.

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Asenjo, A. B., Rim, J., and Oprian, D. D. Molecular Determinants of Human Red/Green Color Discrimination. (1994) *Neuron* **12**, 1131-1138.

Rim, J., and Oprian, D. D. Constitutive Activation of Opsin: Interaction of Mutants with Rhodopsin Kinase and Arrestin. (1995) *Biochemistry* **34**, 11938-11945.

Yu, H., Kono, M., McKee, T. D., and Oprian, D. D. A General Method for Mapping Tertiary Contacts Between Amino Acid Residues in Membrane-Embedded Proteins. (1995) *Biochemistry* **34**, 14963-14969.

Oprian, D. D. Activating Mutations in Rhodopsin. (1996) in *7TM Receptors: Structure and Function in Drug Research. Proceedings of the 39th Alfred Benzon Symposium* (Schwartz, T. W., Hjorth, S. A., Kastrup, J. S., Krogsgaard-Larsen, P., Eds), pp 61-69. Munksgaard, Copenhagen.

Rao, V. R., and Oprian, D. D. Activating Mutations of Rhodopsin and Other G Protein-Coupled Receptors. (1996) *Annu. Rev. Biophys. Biomol. Struct.* 25, 287-314.

Kochendoerfer, G. G., Wang, Z., Oprian, D. D., and Mathies, R. A. Resonance Raman Examination of the Wavelength Regulation Mechanism in Human Visual Pigments. (1997) *Biochemistry* 36, 6577-6587.

Rim, J., Faurobert, E., Hurley, J. B., and Oprian, D. D. *In Vitro* Assay of Trans-Phosphorylation of Rhodopsin by Rhodopsin Kinase. (1997) *Biochemistry* 36, 7064-7070.

Yang, T., Snider, B. B., and Oprian, D. D. Synthesis and Characterization of a Novel Retinylamine Analog Inhibitor of Constitutively Active Rhodopsin Mutants Found in Patients with Autosomal Dominant Retinitis Pigmentosa. (1997) *Proc. Natl. Acad. Sci. USA* 94, 13559-13564.

Kono, M., Yu, H., and Oprian, D. D. Disulfide Bond Exchange in Rhodopsin. (1998) *Biochemistry* 37, 1302-1305.

Kono, M., and Oprian, D. D. "Split Receptors" as a Tool for Analyzing GPCR Structure. (1999) in *Receptor Biochemistry and Methodology* (Series Eds. C. Strader and D. Sibley) *Vol. III, Structure/Function of G-Protein Coupled Receptors* (Wess, J., Ed.). pp. 109-119. John Wiley & Sons, Inc., New York, NY.

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Yu, H., Kono, M., and Oprian, D. D. State-dependent Disulfide Cross-linking in Rhodopsin. (1999) *Biochemistry* 38, 12028-12032.

Yu, H., and Oprian, D. D. Tertiary Interactions between Transmembrane Segments 3 and 5 Near the Cytoplasmic Side of Rhodopsin. (1999) *Biochemistry* 38, 12033-12040.

Ferrer, M., Kapoor, T., Strassmaier, T., Weissenhorn, W., Skehel, J. J., Oprian, D. D., Schreiber, S., Harrison, S. C., and Wiley, D. C. A Biased Combinatorial Approach to Finding Inhibitors for gp41-Mediated Human Immunodeficiency Virus Type-1 Cell Entry. (1999) *Nature Structural Biology* 6, 953-960.

Fasick, J., Ning, L., and Oprian, D. D. Spectral Tuning in the Human Blue Cone Pigment. (1999) *Biochemistry* 38, 11593-11596.

Struthers, M., and Oprian, D. D. Mapping Tertiary Contacts between Amino Acid Residues within Rhodopsin. (2000) *Methods Enzymol.* 315, 130-143.

Struthers, M., Yu, H., and Oprian, D. D. G Protein-Coupled Receptor Activation: Analysis of a Highly Constrained, "Straightjacketed" Rhodopsin. (2000) *Biochemistry* 39, 7938-7942.

Zhou, G., Ferrer, M., Chopra, R., Kapoor, T. M., Strassmaier, T., Weissenhorn, W., Skehel, J. J., Oprian, D. D., Schreiber, S. T., Harrison, S. C., and Wiley, D. C. The Structure of an HIV-1 Specific Cell Entry Inhibitor in Complex with the HIV-a gp41 Trimeric Core. (2000) *Bioorg. and Med. Chem.* 8, 2219-2227.

Ma, J.-X., Kono, M., Xu, L., Das, J., Ryan, J. C., Hazard, E. S., III, Oprian, D. D., and Crouch, R. K. Lack of a Significant Spectral Shift between the A1 and A2 Pigments. (2001) *Vis. Neurosci.* 18, 393-399.

Ma, J.-X., Znoiko, S., Othersen, K. L., Ryan, J. C., Das, J., Isayama, T., Kono, M., Oprian, D. D., Corson, D. W., Cornwall, M. C., Cameron, D. A., Makino, C. L., and Crouch, R. K. Identical Visual Pigment in Salamander Green Rods and Blue-Sensitive Cones. (2001) *Neuron* 32, 451-461.

Abdulaev, N. G., Strassmaier, T., Ngo, T., Chen, R., Cartailler, J.-P., Lueke, H., Oprian, D. D., and Ridge, K. D. Grafting Segments from the Extracellular Surface of CCR5 onto the Transmembrane Helices of Bacteriorhodopsin Confers HIV-1 Coreceptor Activity and Alters the Folding Pathway. (2002) *Structure* 10, 515-525.

Fasick, J., Applebury, M. L., and Oprian, D. D. Spectral Tuning in the Mammalian Short-Wavelength Sensititve Cone Pigments. (2002) *Biochemistry* 41, 6860-6865.

Jin, S., Cornwall, M. C., and Oprian, D. D. Opsin Activation as a Mechanism of Congenital Night Blindness. (2003) *Nature Neuroscience* 6, 731-735.

Xie, G., Gross, A. K., and Oprian, D. D. An Opsin Mutant with Increased Thermal Stability. (2003) *Biochemistry* 42, 1995-2001.

Gross, A. K., Xie, G., and Oprian, D. D. Slow Binding of Retinal to Rhodopsin Mutants G90D and T94D. (2003) *Biochemistry* 42, 2002-2008.

Gross, A. K., Rao, V. R., and Oprian, D. D. Characterization of Rhodopsin Congenital Night Blindness Mutant T94I. (2003) *Biochemistry* 42, 2009-2015.

Oprian, D. D. Phototaxis, Chemotaxis, and the Missing Link. (2003) *Trends Biochem. Sci.*28, 167-169.

Jin, S., McKee, T. D., and Oprian, D. D. An Improved Rhodopsin/EGFP Fusion Protein for Use in the Generation of Transgenic *Xenopus laevis* (2003) *FEBS Lett.* 542, 142-146.

Das, J., Crouch, R. K., Ma, J.-X., Oprian, D. D., and Kono, M. The Role of the 9-Methyl Group of Retinal in Cone Visual Pigments. (2004) *Biochemistry* 43, 5532-5538.

Kim, J.-M., Altenbach, C., Kono, M., Oprian, D. D., Hubbell, W. L., and Khorana, H. G. Structural Origins of Constitutive Activation in Rhodopsin: Role of the K296/E113 Salt Bridge. (2004) *PNAS* 101, 12508-13.

Kono, M., Crouch, R. K., and Oprian, D. D. A Dark-Active and Constitutively Active Mutant of the Tiger Salamander UV Pigment. (2005) *Biochemistry* 44, 799-804.

Tam, B. M., Xie, G., Oprian, D. D., and Moritz, O. L. Mislocalized rhodopsin does not require activation to cause retinal degeneration or neurite outgrowth in X. laevis. (2005) J. Neurosci., in press.

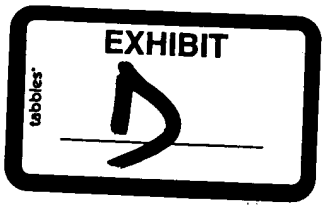
CONGRATULATIONS

ANDREW LEITZ

THE PROTEIN SOCIETY ANNUAL

ELI LILLY POSTER AWARD

PROTEIN SOCIETY 17TH ANNUAL SYMPOSIUM
JULY 26 - 30, 2003 BOSTON, MA



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/990/087
Confirmation No.:		1280
Applicant	:	Sligar et al.
Filed	:	November 20, 2001
TC/A.U.	:	1646
Examiner	:	Ruixian LI
For	:	MEMBRANE SCAFFOLD PROTEINS
Docket No.	:	87-00
Customer No.	:	23713

DECLARATION OF ROGER W. VANHOY

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Roger W. VanHoy, declare as follows:

1. I have been employed as a Technology Manager in the Office of Technology Management at the University of Illinois since July 2001.

2. I estimate that there are approximately 1200 University of Illinois faculty members whose work might result in a request for a material transfer. My responsibilities include review of requests for Material Transfer Agreements from academic and commercial entities outside this university. A Material Transfer Agreement is a legally binding contract which sets forth the terms and conditions related to the transfer of a material from the university to an external requesting party, who may be a representative of an academic or research institution or a commercial entity. Legally authorized representatives of both the university and the external party must sign the document before any material is provided to the requesting party.

3. In the past 28 months, there have been a total of 223 Material Transfer Agreements fully executed for the transfer of university-owned material and technology to third parties. Of these 223, 33 are related to artificial membrane scaffold proteins as described in the present application (or subsequent related applications) or nanoscale disc-like particles containing such artificial membrane scaffold proteins. There are four additional related agreements which have not yet been finalized and signed by both parties

4. In my opinion and experience, the numerous requests for materials related to the above-referenced application represent a significant (and disproportionate) percentage of the requests for university materials processed by the Office of Technology Management, and the relative frequency of these requests reflects a need in the art for the materials related to the above-identified patent application.

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/11/2005
Date

Roger W. VanHoy

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/990/087
Confirmation No.:		1280
Applicant	:	Sligar et al.
Filed	:	November 20, 2001
TC/A.U.	:	1646
Examiner	:	Ruixian LI
For	:	MEMBRANE SCAFFOLD PROTEINS
Docket No.	:	87-00
Customer No.	:	23713

DECLARATION OF ROGER W. VANHOY

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Roger W. VanHoy, declare as follows:

1. I have been employed as a Technology Manager in the Office of Technology Management at the University of Illinois since July 2001.

2. I estimate that there are approximately 1200 University of Illinois faculty members whose work might result in a request for a material transfer. My responsibilities include review of requests for Material Transfer Agreements from academic and commercial entities outside this university. A Material Transfer Agreement is a legally binding contract which sets forth the terms and conditions related to the transfer of a material from the university to an external requesting party, who may be a representative of an academic or research institution or a commercial entity. Legally authorized representatives of both the university and the external party must sign the document before any material is provided to the requesting party.

3. In the past 28 months, there have been a total of 223 Material Transfer Agreements fully executed for the transfer of university-owned material and technology to third parties. Of these 223, 33 are related to artificial membrane scaffold proteins as described in the present application (or subsequent related applications) or nanoscale disc-like particles containing such artificial membrane scaffold proteins. There are four additional related agreements which have not yet been finalized and signed by both parties

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11/11/2005
Date

Roger W. VanHoy